

**Claims**

What is claimed is:

5           1.       A method of isolating cells comprising,  
                 (a) obtaining a tissue sample from a subject,  
                 (b) successively exposing the tissue to a first solution with decreasing  
                 amounts of  $\text{CaCl}_2$  comprising  $\text{NaCl}$ , HEPES,  $\text{MgCl}_2$ ,  $\text{KCl}$ , and sugar at a pH of  
                 approximately 7.4,  
10           (c) disassociating the tissue with an enzyme solution,  
                 (d) repeatedly resuspending the disassociated tissue into a second  
                 solution with increasing amounts of  $\text{CaCl}_2$  comprising Earle's modified salt, L-  
                 glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid,  
                 HEPES, fetal bovine serum, an antibiotic, and a fatty acid, at a pH of approximately 7.4  
15           to obtain isolated cells.

20           2.       The method of claim 1, further comprising the step of re-  
                 suspending the isolated cells approximately every 24 hours in a solution comprising  
                 Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine,  
25           taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid acid, and  
                  $\text{CaCl}_2$  at a pH of approximately 7.4.

30           3.       The method of claim 1, further comprising the step of incubating  
                 the isolated cells in a mixture of carbon dioxide and air.

35           4.       The method of claim 3, wherein the isolated cells are incubated at  
                 approximately 37°C.

40           5.       The method of claim 1 wherein, the first solution is exposed to the  
                 tissue at approximately 37°C and at approximately 4 ml/min for 3 minutes.

45           6.       The method of claim 1 wherein the concentration of  $\text{CaCl}_2$  in the  
                 first solution decreases.

50           7.       The method of claim 1 wherein the first solution comprises  
                 approximately 140 mM  $\text{NaCl}$ , approximately 10 mM HEPES, approximately 1 mM  
                  $\text{MgCl}_2$ , approximately 5.4 mM  $\text{KCl}$ , and approximately 10 mM D-glucose.

8. The method of claim 1 wherein the enzyme solution comprises a digestive enzyme.

9. The method of claim 8, wherein the digestive enzyme is a  
5 protease or a collagenase.

10. The method of claim 1 wherein the concentration of  $\text{CaCl}_2$  in the second solution increases.

11. The method of claim 1 wherein the enzyme solution comprises approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM MgCl<sub>2</sub>, approximately 5.4 mM KCl, and approximately 10 mM D-glucose.

12. The method of claim 1 wherein the second solution comprises  
15 Earle's modified salt, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, Ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, a fatty acid at approximately 1  $\mu$ M at a pH of approximately 7.4.

13. A method of isolating cells comprising,  
(a) obtaining a tissue sample from a subject,  
(b) successively exposing at approximately 37°C the tissue to a first  
solution with decreasing amounts of CaCl<sub>2</sub> comprising approximately 140 mM NaCl,  
25 approximately 10 mM HEPES, approximately 1 mM MgCl<sub>2</sub>, approximately 5.4 mM  
KCl, and approximately 10 mM sugar at a pH of approximately 7.4,  
(c) disassociating the tissue with an enzyme solution for approximately 8  
minutes comprising approximately 140 mM NaCl, approximately 10 mM HEPES,  
approximately 1 mM MgCl<sub>2</sub>, approximately 5.4 mM KCl, and approximately 10 mM  
30 sugar, to form disassociated cells,  
(d) repeatedly resuspending the disassociated cells into a second solution  
with increasing amounts of CaCl<sub>2</sub> comprising Earle's modified salt, L-glutamine,  
sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at  
approximately 328 mg/500ml, taurine at approximately 312mg/500ml, ascorbic acid at  
35 approximately 8.8 mg, HEPES at approximately 2.383g/500ml, fetal bovine serum at  
approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at  
approximately 1 µM at a pH of approximately 7.4 to form a solution of isolated cells,

(e) incubating the isolated cells in a mixture of carbon dioxide and air at approximately 37°C, and

5 (f) re-suspending the isolated cells approximately every 24 hours in a solution comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and CaCl<sub>2</sub> at a pH of approximately 7.4 to obtain isolated cells.

10 14. A method of cultivating isolated cells comprising, resuspending the isolated cells approximately every 24 hours in a solution comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and CaCl<sub>2</sub> at a pH of approximately 7.4.

15 15. The method of claim 14 wherein the solution comprises sodium bicarbonate at approximately 1250mg/l, creatine at approximately 328 mg/500ml, taurine at approximately 312 mg/500ml, ascorbic acid at approximately 8.8 mg/500 ml, HEPES at approximately 2.383 g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at approximately 1 μM, and approximately 1mM CaCl<sub>2</sub>.

20 16. A cell culture media for cells comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and CaCl<sub>2</sub> at a pH of approximately 7.4.

25 17. The cell culture media of claim 16 wherein the media comprises sodium bicarbonate at approximately 1250mg/l, creatine at approximately 328 mg/500ml, taurine at approximately 312 mg/500ml, ascorbic acid at approximately 8.8 mg/500 ml, HEPES at approximately 2.383 g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, a fatty acid at approximately 1 μM, and approximately 1mM CaCl<sub>2</sub>.

30 18. A method of isolating cells comprising,  
35 (a) obtaining a tissue sample comprising cells from a subject ;  
(b) chopping the tissue;  
(c) incubating the tissue in a first solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and nitrilotriacetic acid;

(d) incubating the tissue in a second solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and a digestive enzyme;

(e) incubating the tissue in a third solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and a digestive enzyme; and

5 (f) centrifuging the tissue to obtain isolated cells .

19. The method of claim 18, further comprising the step of resuspending the isolated cells in a culture media comprising medium M199, BSA, ascorbic acid, taurine, carnitine, creatinine, insulin, and an antibiotic .

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20. The method of claim 19, wherein the culture media further comprises a fatty acid or magnesium.

21. The method of claim 18, wherein the first solution comprises  
15 approximately 1-2  $\mu$ M CaCl<sub>2</sub>, approximately 120mM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96.

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22. The method of claim 18, wherein the second solution comprises approximately 1-2  $\mu$ M CaCl<sub>2</sub>, approximately 30  $\mu$ M NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme.

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23. The method of claim 18, wherein the third solution comprises approximately 1-2  $\mu$ M CaCl<sub>2</sub>, approximately 30  $\mu$ M NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme.

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24. A method of isolating cells comprising,

(a) obtaining a tissue sample comprising cells from a subject ;

(b) chopping the tissue;

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(c) incubating the tissue in a first solution comprising approximately 1-2  $\mu$ M CaCl<sub>2</sub>, approximately 120mM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20,

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approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96;

minutes; (d) shaking the tissue at approximately 37°C for approximately 12

5 (e) bubbling approximately 100% O<sub>2</sub> through the solution;  
6 (f) incubating the tissue in a second solution comprising approximately 1-  
7 2  $\mu$ M CaCl<sub>2</sub>, approximately 30  $\mu$ M NaCl, approximately 5.4 mM KCl 5.4,  
8 approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM  
9 glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml  
0 of a digestive enzyme:

(g) incubating the solution in a third solution comprising third solution comprises approximately 1-2  $\mu$ M  $\text{CaCl}_2$ , approximately 30  $\mu$ M  $\text{NaCl}$ , approximately 5.4 mM  $\text{KCl}$  5.4, approximately 5 mM  $\text{MgSO}_4$ , approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme.

15 glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme; and

(h) centrifuging the tissue to obtain isolated cells.

25. A method of isolating and cultivating human myocardial cells comprising,

20 (a) obtaining a tissue sample comprising myocardial cells from a human  
subject;

(b) chopping the tissue;

(c) incubating the tissue in a first solution comprising:

25  $\mu\text{M}$  calcium, approximately 120mM NaCl, approximately 5.4 mM KCl, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96;

minutes; (d) shaking the tissue at approximately 37°C for approximately 12

30 (e) bubbling approximately 100% O<sub>2</sub> through the solution;  
(f) incubating the tissue in a second solution comprising approximately 1-  
2  $\mu$ M, approximately 30  $\mu$ M NaCl, approximately 5.4 mM KCl, approximately 5 mM  
MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20 mM glucose 20,  
approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a  
35 digestive enzyme;

(g) incubating the solution in a third solution comprising third solution comprises approximately 1-2  $\mu$ M, approximately 30  $\mu$ M NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM MgSO<sub>4</sub>, approximately 5 mM pyruvate, approximately 20

mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 400U/ml of a digestive enzyme;

(h) centrifuging the tissue to obtain isolated cells;

(i) repeatedly resuspending the disassociated cells into a second solution

5 which comprises increasing amounts of CaCl<sub>2</sub>, Earle's modified salt, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at 10 approximately 1  $\mu$ M at a pH of approximately 7.4 to form a solution of isolated cells; and

10 (j) incubating the isolated cells in a mixture of carbon dioxide and air at approximately 37°C.

15 26. A method of isolating and cultivating rodent myocardial cells comprising,

(a) removing the heart of a rodent;

20 (b) perfusing the heart with low calcium Tyrode's solution for approximately 3 minutes;

(c) perfusing the heart with an enzymatic solution for approximately 8 minutes;

(d) perfusing the heart with a low calcium solution for approximately 3 minutes;

(e) removing the ventricles;

25 (f) mincing the ventricles to isolate myocardial cells;

(g) mixing the cells in a low calcium solution;

(h) resuspending the cells in a solution comprising increasing concentrations of calcium; and

(i) resuspending the cells in culture media solution..